

**Small Scale Preparation of Topo I and II Extracts from tissue culture cells (optimized for HeLa cells). Perform all operations using an ice bucket. These enzymes inactivate readily in vitro and are easily proteolyzed**

- 1. From 1-2 100 mm petri dishes, scrape up cells into medium**
- 2. Pellet cells 800 xg for 3 min in the cold.**
- 3. Resuspend Cell pellet in 3-5 ml of ice cold TEMP buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 4 mM MgCl<sub>2</sub>, 0.5 mM PMSF) and disperse clumps by pipetting up and down.**
- 4. Repeat centrifugation step and resuspend in 3 ml of TEMP and disperse as above.**
- 5. Leave on ice 10 min.**
- 6. Dounce in tight fitting homogenizer 6-8 strokes (check for nuclei by phase microscopy).**
- 7. Pellet nuclei by centrifugation at 1500 xg for 10 min. (cold).**
- 8. Resuspend the nuclear pellet in 1 ml of cold TEMP. Optional: Transfer to an eppendorf microfuge tube. Repeat step 7 spin or pellet in Microfuge (at 4°C) for 2 min.**
- 9. Resuspend nuclear pellet in a small volume (no more than 4 pellet volumes) of TEP (same as TEMP but lacking MgCl<sub>2</sub>).**
- 10. Add an equal volume of 1M NaCl, vortex, leave on ice for 30-60 min.**
- 11. Spin in ultracentrifuge at 100,000 xg for 1 hr. (cold). Alternatively, you may be able to spin in the cold in a microfuge at 12-15,000 xg for 15 min. Ultracentrifugation is best, however.**
- 12. The supernatant will contain topo I and II activities. The type I activity can easily be assayed using the Topo I assay kit (see below) and the type II using kDNA and the TopoGEN Topo II assay kit. Usually, 10<sup>7</sup> to 10<sup>8</sup> cells extracted in this way should give large amounts of activity in 1 ul of extract; however, one should titrate over a wide range (from 1:100 dilution in TEMP to as much as 4 ul) to ensure that the reactions are not overloaded. Since the extract contains high salt (0.5M) take care not to poison the reaction with excessive amounts of the extract.**

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